REMARKS

Claims 44-91 were pending, and all have been rejected for obviousness. Since claims 68-91 have been cancelled, rejections of these claims are moot. These claims are cancelled solely to comply with the 5/25 requirement of 37 CFR § 1.75, and Applicant reserves the right to pursue this subject matter in future applications. Claims 44-67 are rejected over U.S. Patent No. 6,673,575 (hereinafter, Franze), in view of U.S. Patent No. 6,274,680 (hereinafter, Schnaar), U.S. Patent No. 6,472,175 (hereinafter, Wood), or Gu and Wang (1997), Abstracts of Papers American Chemical Society 213(1-3): BIOT 106 (hereinafter, Gu 1997). The Examiner has cited two references by Gu et al. (1997) in connection with the rejection. However, Applicant is aware of only one Gu et al. (1997) reference. Applicant will assume that the Examiner meant to also cite Gu and Wang (1998), Biotechnol. Bioeng 58: 642-648 (hereinafter, Gu 1998). Applicant requests clarification if this is not the case.

Interview Summary

Applicant thanks the Examiner for the telephone interview of October 29, 2007. Claim amendments to overcome the obviousness rejections were discussed. The Examiner indicated that amending the claims such that the media and methods were limited to use in CHO cells would be very helpful.

Claim Amendments

All claims are now limited to media or methods to be used in culturing CHO cells. In addition, claims directed to a medium (claims 44, 56, and claims dependent thereon) are limited such that use of the medium to culture CHO cells can increase the sialic acid content of a protein produced by the cells.

Newly Submitted References

An Information Disclosure Statement is submitted herewith. It includes a copy of the "Proprietors Observations" (which are in German) in the currently pending opposition to the European equivalent of Franze, EP 1 036 179, as well as a translation of this document. In these documents, the Proprietor has limited all claims in the Main and

Auxiliary Requests to methods using human cells in view of the data provided in reference "D12," which is an Experimental Report produced by Kirin in its opposition to the Japanese equivalent of Franze. Translation of Proprietor's Observations, page 8 and Main and Auxiliary Requests. Applicant submitted an English translation of "D12" in the Information Disclosure Statement dated December 20, 2006 (*see* Kadoya et al.). Thus, the assignee of Franze has now taken a position in Europe that reflects a view that the data in Kadoya et al. show that the invention described in Franze is not operable when practiced in CHO cells.

Obviousness Rejections

With regard to claims 44-67, the Office Action states that Franze teaches culturing cells in a medium for production of sialylated proteins and also suggests the use of combinations of carbohydrates. Fructose, mannose, and galactose are recited within a list of carbohydrates that Franze states can be used to practice the invention. Franze does not specifically suggest, nor does it exemplify, adding a combination of fructose, galactose, and mannose or adding galactose alone to glucose-containing medium. The Office Action further states that Schnaar, Wood, and Gu (1997 and 1998) disclose the use of N-acetylmannosamine (ManNAc) to control sialylation of proteins. The Office Action further states, "Taking the cited prior art as a whole, it would have been obvious at the time the invention was made to make media and use it for controlling the sialylation of proteins (particularly recombinant) by cells (particularly CHO) in culture wherein the media contains fructose, mannose, galactose, N-acetylmannosamine and any combinations thereof as a matter of routine experimentation for the optimizing of sialylation control. The depth of the prior art is significant and clearly it has established that the selection of sugar, amounts thereof and other normal culture parameters are result effective variables." Office Action, pages 6-7.

I. Graham Analysis

Applicants begin by analyzing the *Graham* elements, that is, (A) the scope and content of the prior art, (B) the differences between the claimed invention and the prior art, (C) the level of skill in the art, and (D) any secondary consideration that may be relevant. *Graham v. Deere*, 383 US 1, 148 USPQ 459 (S.Ct. 1966).

A. Scope and Content of the Prior Art

1. Media effects on protein glycosylation are not always the same in all cell types.

Turning to the scope and content of the prior art, Applicant first asserts that the art teaches that results concerning media effects on protein glycosylation are not necessarily the same in different cell types. This assertion is supported by numerous references of record. For example, Schnaar teaches that the quantity of sialic acid detected in intracellular precursor pools and in glycoproteins or glycolipids subsequent to feeding with sialic acid precursors (including ManNAc) were different in Jurkat (a human cell line) versus NG108-15 (a rodent cell line) cells. Schnaar, Figure 3, Example 2. Similarly, Baker et al. (submitted in the Information Disclosure Statement dated December 20, 2006) show a number of differences between GS-NSO (derived from murine myeloma) and GS-CHO (derived from Chinese hamster ovary) cells. The percentages of N-glycans that are biantennary, triantennary, and tetraantennary differ in GS-NSO and GS-CHO cells. Baker et al., Table 1 and text at pages 191-192. The levels of sialyltransferase activity were also very different in these cell types. Baker et al., pages 193-194. In addition, a shift in the usage of certain forms of sialic acid was observed in both GS-NSO cells and GS-CHO cells with the addition of ManNAc to the medium which was qualitatively similar, but quantitatively very different. Baker et al. Table IV, page 195.

Further, such differences in the nature of protein glycosylation between various kinds of mammalian cells are well known in the art. For example, Jenkins et al. (Nature Biotechnol. 14: 975-81 (1996), submitted with the Information Disclosure Statement dated April 10, 2006) describe the choice of an expression system as a crucial one in optimizing cell culture processes for producing a glycoprotein with appropriate glycosylation. With respect to mammalian cells, they comment, "Species that are phylogenetically closer to humans may be expected to have more elements of the glycosylation machinery in common. Nevertheless, there are some surprising differences between the glycosylation characteristics of the rodent cell lines (routinely used for glycoprotein synthesis) and human tissues." Specific differences in the glycosylation machinery of CHO cells versus human cells are discussed. For example, CHO cells lack

an α2,6-sialyltransferase enzyme, which is present in human cells, leading to differences in sialylation. Thus, protein glycosylation varies in different kinds of mammalian cells, a situation that was well-understood in the art as of the priority date of the instant application.

Further references suggest that the particular experimental protocols described in Franze do not yield consistent results in different cell types. Comparison of the experimental results reported in Franze with those reported in Kadoya et al. (*see* the Information Disclosure Statement dated December 20, 2006) suggests that CHO cells and human cells do not behave similarly when subjected to the experimental protocol described in Franze, an interpretation which the Patent Proprietor at least tacitly supports given the position that has been taken in Europe. *See* "Proprietors Response," page 8 and the Main and Auxiliary Requests, submitted with the attached Information Disclosure Statement. Therefore, Applicant asserts that differences exist between different kinds of cells with respect to their protein glycosylation machinery which can lead to differences in the effects of medium additives, such as sugars, on protein sialylation.

Because of these cell-specific differences, Applicant submits that references concerning cell types other than CHO cells are simply not relevant to the claimed invention. Therefore, Applicant asserts that Schnaar, Wood, and Franze are not relevant to the claimed invention because the experiments described therein do not concern CHO cells. Thus, Applicant submits that the teachings of these references are not relevant to the pending claims.

2. Uncertainty in the art regarding the effects of ManNAc on protein sialylation.

Applicant asserts that conflicting data existed in the art concerning whether adding ManNAc to culture medium of CHO cells could increase protein sialylation. Applicant agrees that the two cited Gu references suggest that addition of ManNAc to medium can increase protein sialylation in CHO cells. However, Baker et al. did not observe any significant increase in protein sialylation with addition of ManNAc to the medium of CHO cells. Baker et al., Tables II and III, text at pages 194-195. Thus, Applicant asserts that the art did not teach that addition of ManNAc to CHO cell medium would predictably lead to protein sialylation.

Should the Examiner believe, contrary to Applicant's arguments made above, that results with cell types other than CHO cells are relevant to the pending claims, additional references also indicate that addition of ManNAc to culture medium does not necessarily lead to increases in protein sialylation. Hills et al. (on page 244; *see* Information Disclosure Statement dated December 20, 2006) show that sialylation of an N-glycan in an Fc portion of an antibody did not occur upon addition of ManNAc to culture medium of GS-NSO cells producing the antibody, in spite of a 13-fold increase in CMP-sialic acid, the sialic acid donar for protein sialylation. Similarly, Mantey et al. (Table 1; Figure 1; and text pages 81-82; *see* attached Information Disclosure Statement) observed no increase in cell surface sialic acid expression in normally-sialylated HL60-II cells when fed with ManNAc, although ManNAc addition did increase cell surface sialylation in a hyposialylated HL60 subclone, HL60-I. Thus, these references teach that adding ManNAc to a culture does not necessarily increase sialylation of a protein produced by the cultured cells or increase overall expression of sialic acid on the cell surface.

3. One reference demonstrates that fructose, mannose, and/or galactose are without effect on protein glycosylation in CHO cells.

Nyberg (Nyberg (1998), Ph.D. Thesis, MIT, submitted in Information Disclosure Statement filed December 20, 2006) contains data showing that addition of mannose, galactose, fructose, or a combination of galactose and mannose to medium does not affect N-glycan site occupancy of interferon gamma produced by cultured CHO cells. Nyberg (1998), Ph.D. Thesis, MIT, at page 166 and Figure 6.2. Although protein sialylation was not directly measured in this case, these data do indicate that there is no gross change in at least one aspect of glycosylation with the addition of these sugars.

4. Effects of combinations of medium components cannot be predicted from the effects of single medium components.

Applicant submits that cell culture is an unpredictable art and that therefore the effects of combinations of medium components cannot be reliably predicted from the effects of single medium components. Submitted in the attached Information Disclosure Statement is Chun et al. (2003), Biotechnol. Prog. 19: 52-57. Chun et al. performed a full factorial set of experiments to determine which of four different growth factors, or

combinations thereof, were effective in promoting growth of CHO cells. They found that two of the four growth factors tested were effective in promoting cell growth and protein production. The effects of those two, IGF-1 and recombinant insulin, were not additive. In fact, the combination of the two gave results similar to using one or the other. Chun et al., pages 53-54, Table 1. A variety of explanations for such a result are possible. For example, the authors suggest that both growth factors may signal through the same receptor. Chun et al., page 56. This is but one example of the many kinds of unpredictable interactions possible in a biological system.

5. Feeding cultures with biosynthetic precursors of protein sialylation fails to increase protein sialylation in some cases.

The following example clearly shows that merely feeding cells with biosynthetic precursors of protein sialylation does not reliably lead to increases in protein sialylation. Baker et al. teach that feeding NSO and CHO cells with glucosamine (GlcN) plus uridine (Urd) led to increases (which differed quantitatively in the two cell lines) in levels of intracellular UDP-hexosamines and CMP-sialic acids. Baker et al., page 195. Nonetheless, protein sialylation in these cells was slightly decreased, which is surprising since CMP-sialic acid is the sialic acid donar for protein sialylation. Baker et al., Table III, text page 196. Thus, the feeding of precursors of protein sialylation (of which glucosamine in one), even if it leads to elevation of CMP-sialic acid levels, does not guarantee increases in protein sialylation.

6. The art of cell culture is unpredictable.

In addition, Applicant submits that cell culture in general is unpredictable. One article states: "The influences of cell culture process (including the effects of scale-up) are not as well defined at present, but as more studies are published generic protocols may emerge to produce consistent (albeit heterogeneous) glycosylation patterns." Jenkins et al. (1996), Nature Biotechnology 14: 975-81, at 979.

B. Differences Between the Claimed Invention and the Prior Art

1. Differences if only references concerning CHO cells are considered relevant.

As argued above, Applicant submits that only references utilizing CHO cells are relevant to the invention as presently claimed. If the Examiner agrees that this is the case, the only relevant references cited are Gu (1997) and Gu (1998). Neither of these references teach the use of galactose or galactose, fructose, and mannose in medium to increase protein sialylation. Thus, these references do not teach all claim elements. Therefore, the pending claims are not obvious over these references.

2. Differences if all references are considered relevant.

If the Examiner does not agree that only CHO references are relevant, the difference between the claims and the cited references is that the specific claimed combinations of (1) galactose, mannose, fructose, and ManNAc and (2) galactose and ManNAc are not pointed to in any cited reference. There is a total absence of data concerning the effects of galactose, mannose, and fructose as single components in the cited references, merely conclusory statements in Franze that one or more of these components, which are disclosed as members of a large class including all carbohydrates, could be used to feed a culture to control glycosylation. Franze, col. 3, lines 4-20. Moreover, the data presented in Franze has been shown to be irreproducible in CHO cells. Kadoya et al. In addition, the declaration Dr. Carole Heath under 37 CFR § 1.132 (hereinafter, "Heath Declaration", submitted with the response dated December 20, 2006) indicates that one of skill in the art would not believe that the results in Examples 5 and 7 of Franze were necessarily due to feeding with multiple carbohydrates because the experimental controls, as described, were inadequate to enable one of skill in the art to make such a conclusion. Thus, the teachings of Franze would have no predictive value to one of skill in the art, especially in light of the amendments limiting the claims to use in CHO cells.

Moreover, as argued at some length in Applicant's responses dated October 11, 2005, April 10, 2006, and December 20, 2006, galactose, fructose, and mannose are disclosed in Franze as members of a large genus, including all carbohydrates and all

combinations thereof. Specifically called out carbohydrates include all monosaccharides and disaccharides (estimated to include more than 8.8×10^{12} different combinations in Applicant's response of April 10, 2006) and eleven specific carbohydrates, including mannose, galactose, and fructose, (calculated to include 2047 different combinations in Applicant's response dated April 10, 2006). Thus, the claims have selected specific combinations from this huge genus with no guidance in the form of relevant data in Franze.

C. The Level of Skill in the Art is High

The Heath Declaration discusses the qualifications and competencies of one of skill in the art of designing cell culture processes. Based on the Heath Declaration, Applicants submit that one of skill in the art "would be able to read, understand, and evaluate scientific publications and apply this knowledge to the design of processes. One of skill in the art would have knowledge of and would appreciate basic principles of scientific analysis, including the meaning and importance of a "control" sample, as compared to an "experimental" sample." Heath Declaration, pages 1-2. Thus, one of skill in the art would not simply take everything published on faith, but would evaluate the published data to determine whether the conclusions are well-founded and based on scientific principles. Completely unsupported assertions, such as those in Franze, would thus be given little weight by one of skill in the art.

D. Weighing of Graham Factors

Claims 44-67 are nonobvious under *Graham* for the following reasons. The art taught that addition of ManNAc to culture medium led to increases protein sialylation in some cases and not in others. In fact, two references by the same authors are cited for the proposition that addition of ManNAc can increase protein sialylation, and Applicant has pointed to several other references showing that this is not always the case. Hence the art did not provide a reasonable expectation that addition of ManNAc to culture medium would increase protein glycosylation. This is consistent with data in Example 1 and Figure 2 of the specification in which combinations of either fructose or mannose with ManNAc were shown to produce decreases, not increases, in sialylation. Further, if references not using CHO cells are excluded as irrelevant, no cited reference teaches

using galactose, fructose and/or mannose to increase protein sialylation. Thus, not all claim elements are disclosed by the cited art. If, on the other hand, Franze is considered a relevant reference, Applicants submit that one of skill in the art would not read Franze to indicate that medium containing galactose or the combination of galactose, mannose, and fructose would be effective to increase the sialylation of a protein produced by CHO cells cultured in that medium for the reasons spelled out in the Heath Declaration. Simply put, Franze contains no data indicating that galactose, fructose, and mannose, as single medium additives, would lead to increased protein sialylation. Rather, Franze includes conclusory pronouncements that all carbohydrates and all combinations thereof can be added to cell cultures to control protein glycosylation, which would be accorded little weight by one of skill in the art. In addition, the experiments described in Franze have been demonstrated not to be reproducible in CHO cells. Kadoya et al. Moreover, the claimed combinations are selections out of a huge genus of carbohydrates disclosed in Franze. In view of the unpredictability of the art, the inoperability of the experiments described in Franze in CHO cells, a selection of specific species from the huge genus of Franze is nonobvious.

Rationales to Support Obviousness Post-KSR

New guidance has been set forth for evaluating obviousness post-KSR in 72 FR § 57528-57535. To support a rejection under a rationale that prior art elements have been combined according to known methods to yield predictable results, the following findings must be articulated: 1) the prior art included all elements claimed; 2) one of ordinary skill in the art could have combined the elements as claimed by known methods, and each element would perform the same function it did separately; 3) one of skill in the art would recognize that the results of the combination were predictable; and 4) any other finding believed to be necessary under *Graham*. Under this standard, Applicants submit that the claims are nonobvious as explained below.

With respect to the first point, if only art containing data using CHO cells is considered, the art does not include all the elements of the claims because Franze is the only cited reference teaching the use of fructose, mannose, and/or galactose. For this reason alone, the claims are nonobvious. If Franze is, nonetheless, considered relevant,

the selection of specific combinations from the huge genus of carbohydrates disclosed in Franze is also nonobvious for the reasons explained below.

With regard to the second point, Applicant submits that no cited reference, even if Franze is considered, teaches the effects of galactose, fructose, or mannose, separately, on protein sialylation. Therefore, the claims are not a combination of elements already known to increase protein sialylation, but, rather, a combination of elements with unknown (galactose, fructose, and mannose) or variable (in the case of ManNAc) effects on protein sialylation. Moreover, data within the instant application (Example 1, Figure 2) indicate that mannose and fructose, as single medium additives, are without effect on protein sialylation. Similarly, the combinations of mannose plus ManNAc and fructose plus ManNAc exhibit lower levels of protein sialylation than control cultures.

With regard to the third point, one of skill in the art would not have found the results in the application predictable. First, the influence on ManNAc on protein sialylation in CHO cells was known to be variable. Secondly, the effects of galactose, mannose, and fructose, as single medium additives, on protein sialylation in CHO cells were not known. Thus, there was no basis in the art for supposing that the combination of galactose plus ManNAc or of galactose, mannose, fructose, plus ManNAc would be particularly advantageous.

Moreover, data in the application shows that each element did not perform the same function it did separately, that is, effects were more than additive. Turning to Figure 2 of the instant application, these data indicate that a combination of fructose and ManNAc or of mannose and ManNAc led to decreases in protein sialylation of slightly more than 10% each (relative to a control culture containing only glucose) and that a combination of galactose and ManNAc led to an increase in protein sialylation of slightly more than 20%. Averaging these data together would lead one to suppose that a combination of fructose, galactose, mannose, and ManNAc would have approximately the same amount of protein sialylation as the control culture. Instead, an approximate increase of 45% was observed. Thus, the effects observed with this combination were more than additive and were therefore unexpected and unpredictable. Similarly, addition of galactose alone leads to an increase in protein sialylation of approximately 15%. Combination of galactose with ManNAc led to increases in protein sialylation of over 20%. Although a culture with only ManNAc added was not tested, the results of the

cultures containing fructose plus ManNAc or mannose plus ManNAc, i.e., a decrease in protein sialylation, indicate that the addition of ManNAc does not guarantee an increase in protein sialylation. Thus, the additional protein sialylation observed with the combination of galactose and ManNAc was nonadditive and unpredictable.

To summarize, the claims are nonobvious for the following reasons: the relevant prior art did not contain all claimed elements; each element did not perform the same function in combination as it did separately; and the results were not predictable. Thus, Applicant submits that the claims are nonobvious under this new guidance or under *Graham* and respectfully request notice to that effect.

Conclusion

Applicant submits that all claims are in condition for allowance and respectfully requests notice to that effect. If the Examiner believes that any outstanding issue can be most easily resolved via teleconference, he is invited to telephone the undersigned at the direct dial number listed below.

Respectfully submitted,

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October 29, 2007

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